Figure E1

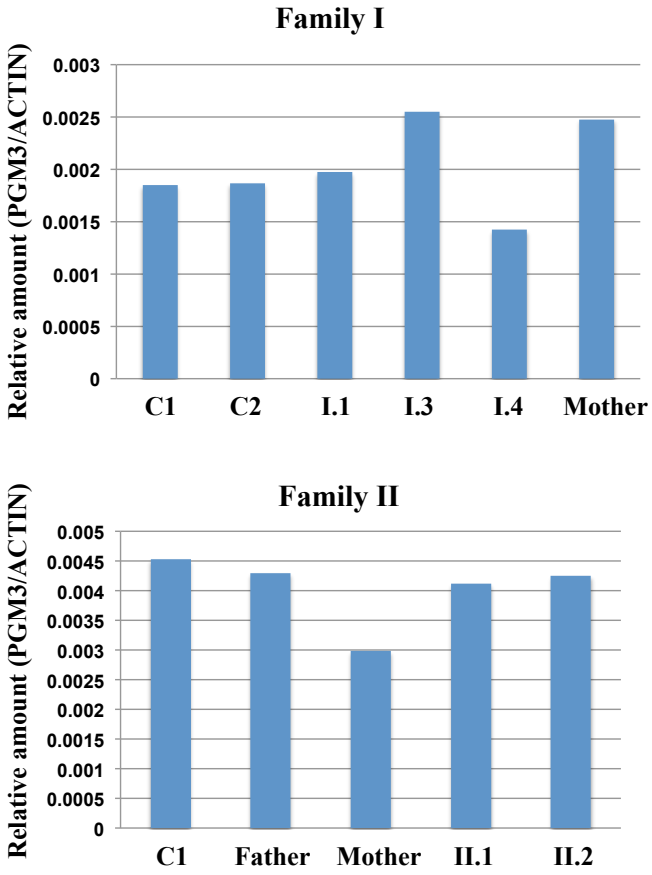

Family I
Relative amount (PGM3/ACTIN)
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
C1
C2
I.1
I.3
I.4
Mother
Family II
Relative amount (PGM3/ACTIN)
0.005
0.0045
0.004
0.0035
0.003
0.0025
0.002
0.0015
0.001
0.0005
0
C1
Father
Mother
II.1
II.2

Figure E2

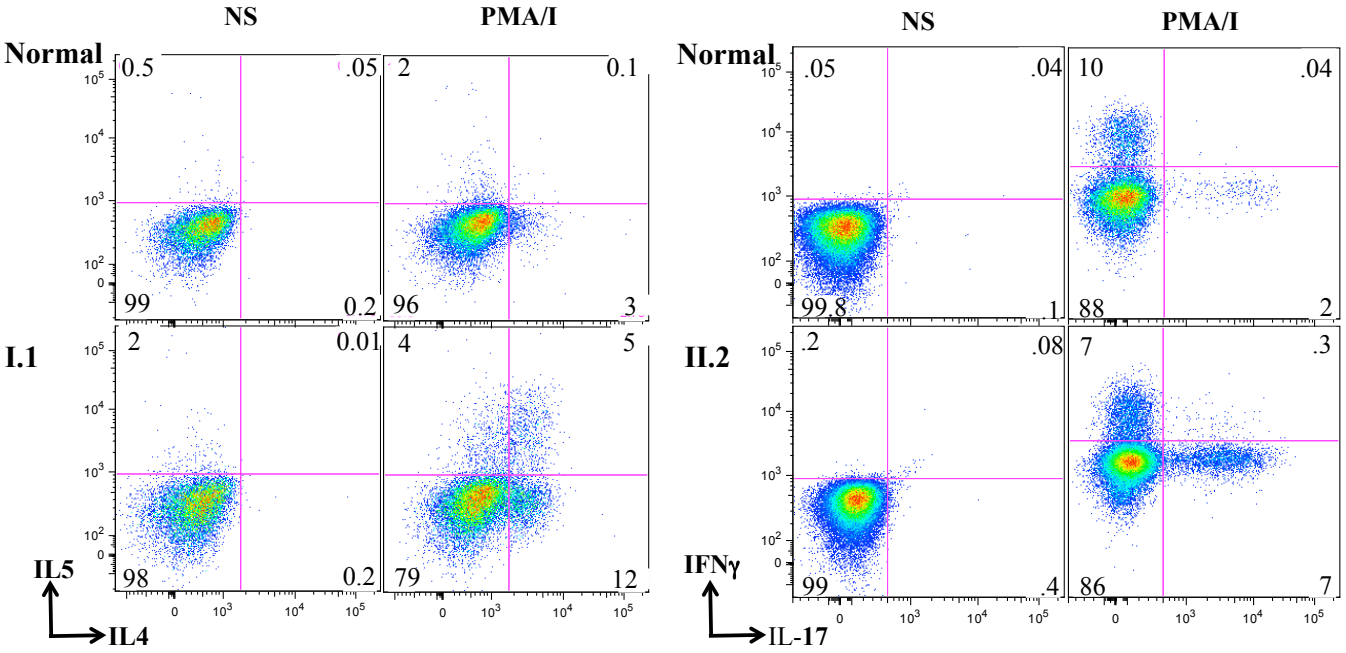
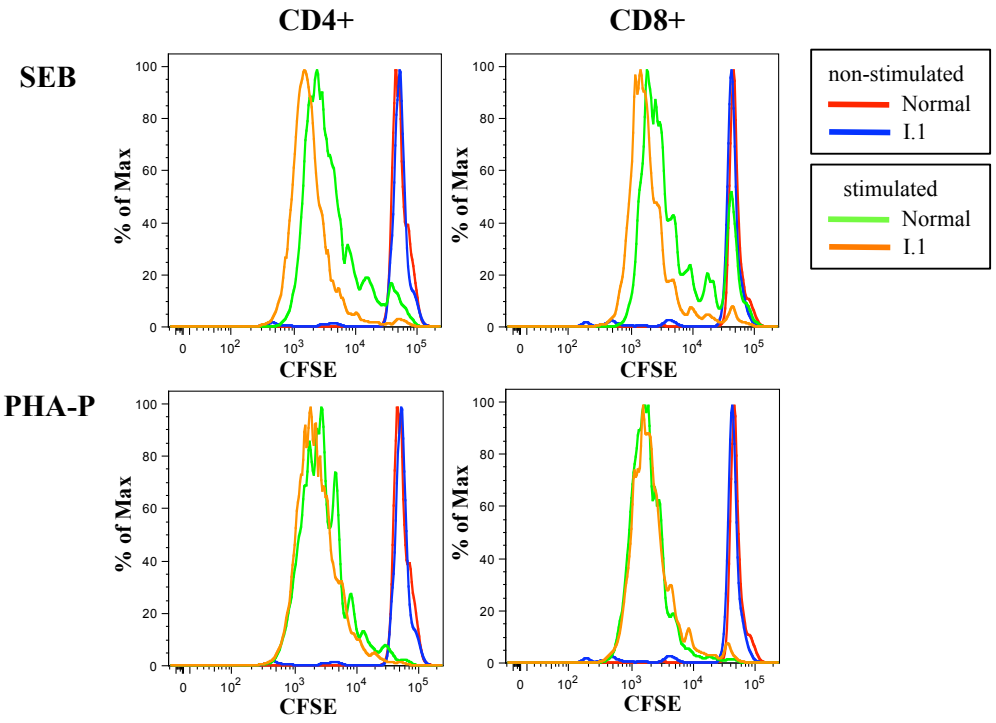

NS
PMA/I
Normal
I.1
0.5
.05
2
0.1
99
0.2
96
3
2
0.01
4
5
98
0.2
79
12
IL5
IL4
NS
PMA/I
Normal
II.2
.05
.04
10
.04
99.8
.1
88
2
.2
.08
7
.3
99
.4
86
7
IFNγ
IL-17

Figure E3


CD4+
CD8+
SEB
PHA-P
% of Max
CFSE
non-stimulated
Normal
I.1
stimulated
Normal
I.1

Online Repository Materials:

METHODS

Genetic analyses

We used a genetics-based approach to identify the molecular defect behind this unusual immunodeficiency with elevated IgE. After obtaining informed consent, DNA samples were collected from either buccal swab or peripheral blood for parents and all four living affected siblings (I.1 to I.4) from Family I, and two affected siblings (II.1 and II.2) from Family II. Whole exome sequencing - SureSelect Human All Exon 50Mb Kit (Agilent Technologies) coupled with Illumina short DNA sequencing - was performed with 3 µg of collected genomic DNA for each sample. About 90-110 million paired-end reads were produced for each sample. All sequenced DNA reads were mapped to the hg19 human genome reference by Burrows-Wheeler Aligner (BWA) with default parameters. Single nucleotide variant and indel calling were performed using the Genome Analysis Toolkit (GATK, http://www.broadinstitute.org/gatk/). The alignments from BWA were recalibrated with dbSNP v134 and the 1000 Genome Project Indel release (http://1000genomes.org). The SNVs/Indels were called by GATK's UnifiedGenotyper from the recalibrated data. Approximately 85,000 SNVs and 12,000 indels were reported from individual samples. After a quality filter based on GATK annotation of strand bias, mapping quality, and SNV cluster, about 65,000 SNVs and 3500 indels remained. All SNVs/indels were annotated by either SeattleSeq Annotation (http://snp.gs.washington.edu) and ANNOVAR (http://www.openbioinformatics.org/annovar/). An in-house custom analysis pipeline was used to process the variant annotation, filter, and prioritize for disease-causal variants.

Briefly, to distinguish potentially disease-causing variants from nonpathogenic variants, we searched for nonsynonymous substitutions or frameshift indels. Existing databases such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) and the NHLBI exome variant server (http://evs.gs.washington.edu/EVS/) have been used to extract previously reported frequency for any given variants and these were used to filter for novel or rare variants. For both families, putative candidate genes were narrowed based on the autosomal recessive inheritance pattern and the consanguinity status of the family. Additionally, SIFT and PolyPhen were used to evaluate each candidate SNV for the possible effect of an amino acid substitution on the structure and function of the respective protein. The expression in immune cells was also used for candidate gene prioritization. WES datasets will be deposited in the dbGaP database.

PCR and DNA sequencing

The coding sequences for *PGM3* were PCR amplified from genomic DNA by using specific primers designed to flank the candidate loci. Amplicons were sequenced on ABI 3700xl DNA Analyzer (Applied Biosystems) according to the manufacturer's instructions.

RT-PCR quantification

The mRNA was isolated from activated T cells by QIAamp RNA Mini Kit (Qiagen) and DNase treated with RNase-free DNase set (Qiagen) according to the manufacture’s protocol. The mRNA was reverse-transcribed using SuperScript III First-Strand Synthesis System (Invitrogen). Gene expression was then quantified by real-time PCR with the Applied Biosystems 7900HT Fast Real-Time PCR System (Applied Biosystems). The mRNA input was normalized to the expression of housekeeping genes (β-actin).

Immunoblot analysis

Standard methods were used to prepare cell lysates with SDS buffer, followed by separation on NuPAGE 4-12% Bis-Tris gels (Invitrogen), transfer onto nitrocellulose membrane, and immunoblotting. Proteins were detected using mouse anti-PGM3 monoclonal antibody (clone 1E2-1B12, No. WH0005238M1; Sigma-Aldrich) or mouse anti–β-actin antibody (Sigma-Aldrich).

PGM3 structural analysis

SWISS-Model (http://swissmodel.expasy.org/) was used to model PGM3 based upon on the structure of the closest available homolog, N-acetylglucosamine-phosphate mutase from *Candida albicans*, which shares ~45% amino acid sequence identity to the human protein. The protein structures of 2DKA (holo-enzyme), 2DKC (substrate-bound enzyme), and 2DKD (product-bound enzyme) were all used to generate comparative models of the enzyme in its different catalytic states. All 3D protein structures were depicted using PyMOL (http://www.pymol.org/).

PGM3 enzyme activity assay

The standard assay mixture contained 50 mM Tris-HCl buffer (pH 8.0), 5 mM $MgCl_2$, 5 mM GlcNAc-6-P, 0.2 mM Glc-1,6-diP, and cell lysate from fibroblasts (25-50 μg of protein) in a final volume of 100 μl. The mixture was incubated at 37 °C for 0-120 min, and at each time point, 5 μl reaction mixture was removed, frozen, and dried. Subsequently dried samples were derivatized with hydroxylamine hydrochloride in pyridine and *N*, *O*-bis[trimethylsilyl]trifluoroacetamide (BSTFA) and subjected to GC-MS analysis to measure the production of GlcNAc 1-P. Standard curve was used to quantify GlcNAc1-P .

Sugar phosphate analysis with GC-MS

Fibroblasts were harvested, extracted twice with 0.1 M acetic acid and sonicated briefly on ice. Supernatants were collected after centrifugation, lyophilized and derivatized in a similar manner to those described in the assay for PGM3 activity section (vide supra) and subjected to GC-MS according to the method described elsewhere (Ichikawa *et al*, manuscript in preparation).

Extraction and analysis of nucleotide sugars

Nucleotide sugars were extracted from fibroblasts and purified by an Envi-Carb carbon column according to the methods by Rabina (Glycoconjugate J 2001). The extracted nucleotide sugars were separated and quantitated by a reverse phase HPLC on an Inertsil ODS-4 column according to the method by Nakajima (Glycobiology 2010). Each nucleotide sugar was idetified by comparison with retention time of corresponding standard. Nucleotide sugar levels were determined based on the peak area relative to ATP.

Complementation with exogenous GlcNAc

Patients' fibroblasts and control fibroblasts were treated with or without 10 mM GlcNAc in the presence of 10% dialyzed serum for 18hr prior to sugar nucleotide analysis.

Flow cytometric analysis of cytokine response

Human peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque gradient separation. 10^6 cells in RPMI 1640 medium supplemented with 10% FBS, 2 mM L-glutamine and 1% penicillin/streptomycin) were stimulated with PMA (Calbiochem, 20 ng/mL), ionomycin (Calbiochem, 1 mM), and Brefeldin A (Sigma-Aldrich, 10 mg/mL) for 6-12 hours at 37 ^{o}C. Non-stimulated (NS) cells were incubated for 6-12

hours at 37 ^{o}C with Brefeldin A alone. After stimulation, intracellular cytokine staining was performed as described previously [1]. Briefly, cells were washed twice with PBS, stained with Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen) for 15 minutes at 4 ^{o}C, and then with the following antibodies: CD45RO TexasRed-PE and CD27 PE-Cy5 (Beckman Coulter), CD8 APC-H7, CD3 AF-700 (BD Pharmingen), or CD3 Q-dot 605 (Invitrogen) for 30 minutes at 4 ^{o}C. Cells were washed once with PBS/0.5% BSA, fixed with 4% paraformaldehyde for 5 minutes at room temperature, and washed two times with PBS/0.5% BSA. Cells were then permeabilized with PBS-Saponin/5% non-fat dry milk for 1 hour or overnight at 4 ^{o}C and stained with the following antibodies: CD4 PE-Cy7, IFN-γ FITC, IL-4 PE, and IL-5 APC (BD Biosciences) or IL-17e660 (eBiosciences) for 30 minutes at 4^{o}C. Cells were washed once with PBS-Saponin and then run on a BD LSR Fortessa. Data was analyzed using FlowJo software (Treestar) by gating on Live^{+} CD3^{+} CD4^{+} CD8^{-} CD45RO^{+} CD27^{-} cells. Statistical significance was calculated from the median value with a two-tailed Mann-Whitney test with GraphPad Prism 6.0 Software.

Flow cytometric analysis of T cell proliferation

The CellTrace CFSE Cell Proliferation Kit (Invitrogen) was used according to the manufacturer's protocol. CFSE-labeled PBMC (200,000) were cultured in a single well of a 96-well round bottom plate in 200 µL of RPMI 1640 medium (5 mM glucose) supplemented with 10% FBS, 2 mM L-glutamine, and 1% penicillin/streptomycin. Cells were stimulated for 4.5 days with SEB (1 µg/mL) or PHA-P (5 µg/mL) (both Sigma-Aldrich). After stimulation, cells were washed twice with PBS, stained with Live/Dead Fixable Aqua Dead Cell Stain Kit (Invitrogen), and then the following antibodies: CD45RO TRPE and CD27 PECy5 (both Beckman Coulter), CD3 Q-dot 605 (Invitrogen),

CD152 PE, CD8 APC-H7, and CD4 PE-Cy7 (all from BD Pharmingen). Samples were run on a BD LSR Fortessa and data was analyzed using FlowJo software.

MRI

MRI scans were performed at various time points during the evaluation using standard clinical protocols on 1.5 and 3.0 tesla systems that were available at the time of examination. T1-weighted, T2-weighted, and T2-FLAIR scans were reviewed by an experienced neuroradiologist (DSR). As these scans were reviewed retrospectively, specific scanning protocols were variable.

FIGURE LEGENDS

Figure E1. Quantitative RT-PCR analysis of PGM3 expression in patients from both families. Levels of PGM3 are shown normalized to β-actin.

Figure E2. Elevated T_H2 and T_H17 cytokines in patients with *PGM3* mutations. Representative dot plots of data shown in Figure 4. $CD3^+$ $CD4^+$ $CD45RO^+$ gated cells producing IL-4, IL-5, IL-17 or IFN-γ in non-stimulated (NS) and PMA and ionomycin (PMA/I) stimulated PBMCs from normal controls and patients.

Figure E3. T cell proliferation of patients with *PGM3* mutations. Total PBMC from normal controls and patients were labeled with CFSE and stimulated with either SEB (1ug/mL) or PHA-P (5ug/mL) for 4.5 days. Cells were gated on $CD4^+$ or $CD8^+$ T cells for flow cytometric analysis. Representative histogram of 2 patients tested in 4-6 different experiments.

1. Foster B, Prussin C, Liu F, Whitmire JK, Whitton JL. Detection of intracellular cytokines by flow cytometry. Curr Protoc Immunol 2007; Chapter 6:Unit 6 24.